FAST AND RELIABLE ONE AND TWO-DIMENSIONAL ELECTROPHORETIC PARAMETERS FOR PHASEOLIN TYPE IDENTIFICATION

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Analysis of phaseolin by 1D-SDS-PAGE [1], and 2D-IEF-SDS-PAGE [2], reveals characteristic profiles of polypeptide subunits due to their microheterogeneity in MW and pI [3].

Up to the present at least 50 types and subvariants of phaseolin have been reported in wild, landrace and improved bean genotypes. Reliable phaseolin type identification is important for detailed studies and understanding of the characteristics of each type. The purpose of this paper is to present modifications of electrophoretic parameters, for 1D-SDS-PAGE and 1D-IEF, which are easier, faster and more reliable than others normally used [4,5] to obtain a clear visual identification of phaseolin type.

With these, it also is possible to determine the MW and pI of each of the ppc of each phaseolin type.

The extraction was made on a 60 mesh bean flour of manually dehulled seeds, with at least 10 seeds per sample. 50 mg of flour was suspended in 500 µl of extractant buffer, 176 mM Tris-HCl, pH 8.5 with 1% NaCl. The vials were placed, immersed in water, in an ultrasound cleaner (American Beauty S/991) during 1 hour without heating, because these conditions were found to result in the maximum total protein extraction. The extract was centrifuged at 10,500 x g (Microfuge-12, Beckman) during 15 minutes and the supernatant recovered. 1D-SDS-PAGE was carried out in a Mini-Protean II apparatus with a 6 cm long and 0.75 mm thick gel. The 0.5 cm stacking gel contained 6.38% T, 2.63% C, 0.1% w/v SDS in 1.17 mM Tris-HCl, pH 6.8 buffer. The separation gel contained 9.82% T with an acrylamide to Bis ratio of 200:1; 0.1% w/v SDS in 9.55 mM Tris-HCl, pH 8.3 buffer. Polymerization was initiated with the addition of 0.05% v/v TEMED and 0.5% v/v of ammonium persulphate from a 10% w/v stock solution. Protein sample extracts were diluted, in equal volumes, in sample buffer; 2.0% w/v SDS, 1.0% 2-mercaptoethanol, 1.80 mM EDTA, 1.17 M sucrose in 0.625 M Tris-HCl, pH 6.8 buffer with 0.01% w/v Bromophenol Blue, and heated to 95°C for 5 minutes before loading onto the gel. 1 µl extract was loaded in each sample well and 200 V were applied to give a starting current of about 150 mA and declining to 50 mA during electrophoresis at constant voltage, during 50 minutes. Gels were stained with Coomasie Brilliant Blue R-250; 0.25% w/v in Methanol:water:acetic acid solution (5:5:1) during 1 hour with gentle shaking (100 rpm), and then destained with Methanol:water:acetic acid solution (30:63:7) during 1 hour with gentle shaking.

1D-IEF was performed based on the method reported by Bollini and Vitale [6] with the following modifications: Slab gels were run in a vertical Mini-Protean II apparatus, with 6.5 cm long gels, 0.75 mm thick, without stacking gel. Gels contained 2.0% ampholite (Serva) pH 5-7, 2.7% T with an acrylamide to Bis ratio of 12.5:1; 8.0 M urea and 0.1% Triton X-100. Polymerization was initiated with the addition of 0.1% v/v TEMED and 0.15% v/v of ammonium persulphate from a 10.0% w/v stock solution. The run was performed at 400 V with the current starting at 20 mA and declining to 2-3 mA during constant voltage

electrophoresis, during 3 hours. 1.0% v/v Phosphoric Acid was used as anolyte (250 ml) plus 25 ml Glacial Acetic Acid. The catholyte was 0.02 M NaOH in 400 ml of degassed water, stored in a plastic bottle. 3µl of each sample, the same used for 1D-SDS-PAGE, were applied to the gel near to the cathode without pre-run. After the electrophoresis the gel was stained directly without equilibration; the staining and destaining were similar for 1D-SDS-PAGE gels. pH gradient in the focused gel was read according to O'Farrel [7] in 0.5 cm wide bands with the carrier dissolved in degassed water.

In the 1D-SDS-PAGE gels the banding pattern of phaseolin types shows between 2 and 6 sharp bands, covering about 1-1.5 cm distance between the ppc with highest and lowest Mr, with 0.5 kD accuracy. This distance and resolution allows the reliable identification of each phaseolin type.

The separation of the ppc of each phaseolin type on the gels is very striking. The MW and pI's of these chains are being established and will appear in a future publication. With regards the IEF-gels, the sharp bands of different phaseolin component ppc cover about 1.5-2 cm distance between the higher and lower pI, with 0.01 pH unit accuracy. The described 1D-IEF and 1D-SDS-PAGE conditions have been combined to generate a fast and reliable 2D-IEF-SDS-PAGE system adequate to identify clearly phaseolin types. The major change for use in two dimensions is the use of capillary tubes instead of slab gel for the first dimension (IEF) separations. The gel tubes are placed on the slab gel of SDS-PAGE, without stacking but covered later with agarose (1% w/v). The system is run in conditions previously described for the PAGE. The silver staining is according O'Farrell [7]. The resolution of the ppc's is high enough for clear identification. The surface area covered in a typical cases for the ppc's is about 3 cm^2, 2 cm on the pI axis and 1.5 cm in the MW axis.

This resolution of ppc permits an adequate quantification by laser scanning densitometry [8], either with moist gels, or with gels dried after treatment with 50:50 ethanol:water solution and covered with celophane before air drying.

The ease with which these new electrophoretic conditions can be applied; combined with significant savings in reagents and time and improved results as presented above make the use of this method recommendable, especilly for fast determination of phaseolin type and the MW and pI's of the component ppc.

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